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Bhattacharai, Mamata

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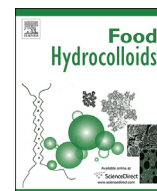
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# Functionality of spruce galactoglucomannans in oil-in-water emulsions

Mamata Bhattarai<sup>a,\*</sup>, Leena Pitkänen<sup>a</sup>, Veikko Kitunen<sup>b</sup>, Risto Korpinen<sup>b</sup>, Hannu Ilvesniemi<sup>b</sup>, Petri O. Kilpeläinen<sup>b</sup>, Mari Lehtonen<sup>a</sup>, Kirsi S. Mikkonen<sup>a</sup>

<sup>a</sup> Department of Food and Nutrition, University of Helsinki, P.O. Box 66, Finland

<sup>b</sup> Natural Resource Institute Finland, Tietotie 2, 02150 Espoo, Finland

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## ABSTRACT

For a sustainable food chain, the demand for plant-based, functional, and cost-effective food hydrocolloids is on a high-rise. Hemicelluloses from the renewable lignocellulosic biomass are available in abundance from side-streams of the forestry industry to fulfill this demand. Their effective valorization requires a safe, economic extraction method that can be up-scaled to an industrial scale and, simultaneously, understanding of their functionality to develop applications. In this study, an aqueous-based extraction method, pressurized hot water extraction (PHWE) of spruce saw meal was used to obtain galactoglucomannans (GGM), “spruce gum”. Ethanol precipitation was performed to remove non-polysaccharide extractives such as free phenolic compounds, and the emulsion component ratio-dependent interfacial saturation capacity of the remaining purified fraction was studied to understand its functionality. GGM resulted in good to excellent emulsification and stabilization of oil-in-water emulsions and exhibited adsorption at the oil droplet interface, which depended on the amount of oil and droplet size of emulsions. The adsorbed GGM content was determined by gas chromatography after acid methanolysis, and their macromolecular characteristics were studied by size-exclusion chromatography. At GGM to oil ratios 2, 1, and 0.4, stable emulsions with predicted several months of shelf life at room temperature were achieved. The results indicated mechanisms affecting the physical stabilization and breakdown of emulsions containing spruce gum, a novel sustainable hydrocolloid.

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## 1. Introduction

The market of food hydrocolloids is growing rapidly, and its value is expected to reach \$7.56 billion by 2020 (MarketsandMarkets, 2016). As consumers are more conscious than ever about health and environment, the demand for innovation and “clean” food products has increased significantly. Hydrocolloids are integral ingredients in a wide range of processed food products and beverages and include polysaccharides from plants, seaweeds, microbes, plant exudates, and modified biopolymers from starch, and cellulose. Most are high-molar-mass hydrophilic biopolymers and are able to stabilize emulsions by modifying the rheological properties of continuous phase. Only a few of them (namely, gum arabic, some pectins, some galactomannans, modified starch, and modified cellulose) function as emulsifiers (Dickinson, 2003). For

example, gum arabic is an established hydrocolloid emulsifier but an expensive ingredient, and replacements such as corn fiber gum, an arabinoxylan (hemicellulose)-rich product from corn milling, are being sought (Yadav, Johnston, Hotchkiss, & Hicks, 2007).

Under the framework of sustainable bioeconomy, a wood-based bioeconomy is envisioned to tackle major global challenges of depleting natural resources in various sectors. As paper consumption has declined over the past years, traditional mills are in the process of becoming biorefineries for the efficient valorization of all lignocellulosic components (Ministry of Economic Affairs and Employment, 2017). Spruce is an industrially important softwood variety for pulping and papermaking process, where cellulosic fibers are the main product, and hemicelluloses and lignin are usually burned for energy. Additionally, sawdust is available as a by-product from sawmills which is currently either burned or used in low-value products. About 25–35 wt% of softwood tissues consist of extractable non-cellulosic polysaccharides (hemicelluloses), mainly acetylated galactoglucomannans (GGM)

\* Corresponding author.

E-mail address: [mamata.bhattarai@helsinki.fi](mailto:mamata.bhattarai@helsinki.fi) (M. Bhattarai).

(Willför, Sundberg, Tenkanen, & Holmbom, 2008). They have a linear backbone of  $\beta$  (1  $\rightarrow$  4) linked D-Manp and  $\beta$  (1  $\rightarrow$  4) linked D-Glcp substituted by the  $\alpha$ -D-Galp residues at the C-6 position, preferably at mannose units that are acetylated at C-2 or C-3 with a degree of substitution of 0.3. GGM with a molar mass ranging from 12,000 to 60,000 g/mol (Mikkonen et al., 2016; Mikkonen, Xu, Berton-Carabin, & Schroën, 2016) have been extracted with different methods; from process water during thermomechanical pulp treatment (Willför, Rehn, Sundberg, Sundberg, & Holmbom, 2003; Xu, Willför, Holmlund, & Holmbom, 2009), pressurized hot water extraction (PHWE) of sawdust (Kilpeläinen et al., 2014) and a pending patent from CH-Bioforce Oy (Von Schoultz, 2015). With an intermediate viscosity in aqueous solutions, and interfacial activity between to that of conventional, amphiphilic, small molecular surfactants and large macromolecular hydrocolloids, GGM is an interesting option that is yet to be commercially utilized.

The abundantly available GGM, or “spruce gum”, can be valorized as novel, functional hydrocolloids in oil-in-water emulsions in pharmaceuticals, cosmetics, paints, and coatings, for example. In previous studies, GGM exhibited higher emulsification and stabilization capacity against both physical breakdown and lipid oxidation compared to the current gold standard polysaccharide hydrocolloid, gum arabic, and its suggested replacement, corn fiber gum (Lehtonen et al., 2016; Mikkonen, Merger et al., 2016; Mikkonen, Xu et al., 2016). In contrast to functionality of most polysaccharide-based emulsifiers that originates from the residual contaminants (e.g., protein fractions in gum arabic and galactomannans) (Dickinson, 2003), in earlier studies, the emulsification and stabilization abilities of GGM were strongly hypothesized as a mixed effect from inter- and intra-molecular polysaccharide assemblies inducing particle-type stabilization (Pickering effect) and the presence of co-extracted phenolic residues in GGM acting as hydrophobic anchor groups inducing steric stabilization. GGM obtained by PHWE method consist of 75 wt% polysaccharides, and the rest are other wood-derived compounds, primarily lignins and extractives (Kilpeläinen et al., 2014). Phenolic residues contribute to but are not alone responsible for the observed stability of GGM emulsions (Lehtonen et al., 2018) thus, the role of the polysaccharide-rich fraction of GGM on the stabilizing functionality remains unresolved.

Polysaccharides form a thick adsorbed layer generating good steric barriers against coalescence in emulsions due to their large molecular volume (Dickinson, 2017) compared to small molecular surfactants (Wilde, Mackie, Husband, Gunning, & Morris, 2004). However higher amounts of polysaccharides are required for the complete saturation of interfaces due to their lower surface activity (McClements, Bai, & Chung, 2017). This result in instability in emulsions from either bridging or depletion flocculation exhibited by different nature of association of droplets – open or closed flocs (McClements, 2007). The former is due to sharing of polysaccharide chains between droplets due to their incomplete saturation that can largely promote coalescence and the latter is due to a high concentration of unadsorbed polysaccharides in the continuous phase (Dickinson, 2003). Similarly, unadsorbed polysaccharides also re-stabilize emulsions if present in sufficiently high concentrations by conferring a very high apparent viscosity to the continuous phase and/or generating a reversible gel network. Recently, they have also been accounted for in their role in stabilization by forming interfacial barriers in the form of aggregates (Dickinson, 2017).

In this study, the PHWE extract of spruce was ethanol-precipitated to remove free phenolic compounds (those not bound with GGM) and other non-polysaccharide extractives. We aim to understand the emulsification and stabilization mechanisms of the polysaccharide-rich fraction of spruce extract through

characterizing the optimal component ratios of GGM stabilized emulsions for desired interfacial saturation. The component ratios in emulsions were correlated with droplet size, interfacial area, surface load, emulsion stability, and breakdown mechanisms using chemical and physical methods. The emulsion stability is discussed in relation to the adsorption of GGM at the interface and to the macromolecular characteristics of distributed polysaccharides.

## 2. Materials and methods

### 2.1. Materials

GGM was extracted using the pressurized hot water extraction (PHWE) process on spruce sawdust (Kilpeläinen et al., 2014). The sawdust was obtained from a sawmill (Herralan Saha, Herrala, Finland). A 96.9 kg (43.5 kg dry weight) spruce sawdust was extracted at 170 °C for 70 min with a 20 kg/min flow rate. A total of 1050 kg of extract was collected. The extract was filtered by an ultrafiltration system (PCI-membranes) using tubular modified polyethersulfone membranes (EM006). Before ultrafiltration, the extract pH was adjusted to neutral with sodium hydroxide to increase permeate flow through the membrane. As pH lowered during the ultrafiltration, NaOH was added to keep the pH of the concentrate neutral for a stable permeate flow. In this way, concentrated GGM was obtained from PHWE extract.

Concentrated GGM was precipitated with ethanol (1/8 concentrate/ethanol v/v) by slowly adding concentrate to an ethanol phase that was mixed thoroughly. When all concentrate was added to the ethanol, the solution was mixed for 15 min and left to precipitate overnight. The ethanol was siphoned away and the precipitated polysaccharide slurry was transferred to a filter bag (Eaton NMO-25-P01R-50 S, Hyxo Oy, Finland) where excess ethanol was removed. The slurry was filtered through a Buchner funnel using a pore 2 cellulose filter (Whatman) followed by drying in a vacuum oven at 40 °C for 2 days. This purified sample consisted mainly of polysaccharides and is referred to as GGM in this study. A summary of the extraction and purification method is illustrated in Fig. 1.

Rapeseed oil was purchased from a supermarket and purified by adsorption chromatography according to the previously described method (Lampi, Dimberg, & Kamal-Eldin, 1999). The oil was stored at –18 °C for further use. The purification was performed to remove any possible surface-active species (e.g., tocopherols) in commercial rapeseed oil to obtain a clear insight into the functional role of polysaccharide entities alone in the dispersed systems.

### 2.2. Reagents

Citric acid monohydrate was from Sigma-Aldrich (St. Louis, MO, USA). Sodium azide and sodium nitrate were from Merck (Darmstadt, Germany). Sodium dodecyl sulfate (SDS) was obtained from VWR's BDH Chemicals (Belgium). All solvents used were HPLC grade. Heptane, methanol, and anhydrous methanol were from Sigma-Aldrich (St. Louis, MO, USA). Pyridine and Bis(trimethylsilyl) trifluoroacetamide (BSTFA) were from Merck (Darmstadt, Germany), and trimethylsilyl chloride (TMSCl) was from Fluka (St. Louis, MO, USA).

Monosaccharide standards D-xylose, D-glucose, D-galactose, D-mannose were from Merck (Darmstadt, Germany), D-galacturonic

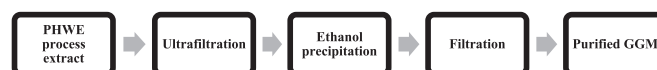


Fig. 1. Schematic diagram of extraction and purification process of GGM.

acid monohydrate was from Fluka (St. Louis, MO, USA), and D-glucuronic acid was from Aldrich (St. Louis, MO, USA). Pullulan standards were obtained from Postnova Analytics (Landsberg am Lech, Germany). Bovine serum albumin (BSA) for protein analysis was from Sigma-Aldrich (St. Louis, MO, USA).

### 2.3. Emulsion preparation

GGM were dissolved in 25 mM citrate buffer (pH 4.5) at room temperature (RT) while stirring with a magnetic stirrer overnight. A 1 wt% GGM was used to emulsify 0.5, 1, 2.5, 5, and 10 wt% rapeseed oil. The emulsions were premixed by an Ultra-Turrax (T-18 basic, IKA, Staufen, Germany) equipped with a disperser-type stirrer at 11,000 rpm for 5 min to obtain coarse dispersions. A high-pressure homogenizer (Microfluidizer 110Y, Microfluidics, Westwood, MA, USA) was used to further break down the coarse oil droplets to fine droplets with three passes at a pressure of 800 bar. The homogenizer was configured with 75  $\mu$ m Y-type F20Y and 200  $\mu$ m Z-type H30Z chambers in a series. A 0.02 wt% sodium azide was added to emulsions after homogenization to prevent microbial spoilage.

### 2.4. Emulsion characterization during storage

The emulsions were stored in a dark space at 40 °C for an accelerated storage test of 9 weeks. Droplet size distribution (DSD) measurement and optical microscopy were performed on emulsions as fresh, after 1 h on the preparation day, and after 1, 2, 4, 6, and 9 weeks of storage. The DSD of the emulsions was determined by the static light scattering technique using a Mastersizer Hydro 3000 SM (Malvern Instruments Ltd, Worcestershire, UK). Refractive indexes of 1.33 for water and 1.47 for dispersed phase were used. The rotor speed during measurement was 2400 rpm. Duplicate sampling and three measurements on each sample were performed. The emulsions were gently turned upside down 10 times before sampling. The surface mean diameter  $D[3,2]$  and volume mean diameter  $D[4,3]$  are reported as mean and standard deviation from a total of six measurements. To indicate the width of the size distribution,  $D[10]$ ,  $D[50]$ , and  $D[90]$  values were also measured. The total interfacial area, number of droplets, and surface load were calculated from the amount of oil in emulsion using the  $D[3,2]$  value after 1 h of preparation. A density of 0.905 g/cm<sup>3</sup> was used for rapeseed oil.

The oil droplets were visualized using an optical microscope (AxioScope A1, Carl Zeiss Inc., Oberkochen, Germany). Samples were mixed in a similar way as described above.

### 2.5. Phase partitioning

After 1 h of preparation, the emulsions were partitioned into cream and continuous phases following the method of Mikkonen, Xu et al. (2016) with some modifications. High-speed centrifugation at 24,000 g for 30 min at RT was used to separate the emulsion phases. The bottom part was collected carefully and centrifuged for an additional 30 min at the same speed. If present, the remaining cream was collected and the continuous phase was lyophilized to obtain the unadsorbed fraction. The total collected cream was weighed and mixed with 1% SDS solution in cream: SDS solution ratio of 1:10 (w/v). SDS, being a more effective surfactant than GGM, can replace GGM from the interface (Chapleau & de Lamballerie-Anton, 2003; Mikkonen, Xu et al., 2016). The mixture was stirred overnight with a magnetic stirrer and centrifuged again for 30 min at 24,000 g. GGM replaced from the interface was collected at the bottom part and lyophilized. The collected fractions from the interface and continuous phase were further mixed with heptane and centrifuged for 5 min at 15,000 g three times to

remove oil residues, followed by drying in a vacuum oven at 25 °C for 1 h and stored for analysis. In this way, adsorbed and unadsorbed fractions from all five emulsions were obtained via phase partitioning.

### 2.6. Polysaccharide analysis

Quantitative analysis of total polysaccharide content of starting GGM (sGGM) and partitioned GGM fractions was performed by analyzing the monosaccharides with gas chromatography (GC) after acid methanolysis and silylation, according to the previously described method (Sundberg, Sundberg, Lillandt, & Holmbom, 1996). The instrument details and method for GC analysis are described in Chong et al. (2013). Quantification was performed using six levels of concentration of each monosaccharide. Methyl glucuronic acid was quantified based on the D-glucuronic acid standard as described in Chong et al. (2013). The total polysaccharide content was calculated from the monosaccharide content of triplicate samples.

### 2.7. Molar mass analysis

Size exclusion chromatography (SEC) with an online combination of a viscometric detector, refractive index (RI), and an ultraviolet (UV) detector at 280 nm was used to study the molar mass distribution of the GGM samples. The instrument details have been previously described (Pitkänen, Tuomainen, Mikkonen, & Tenkanen, 2011). The partitioned GGM and sGGM powder were dissolved in MilliQ water with a 0.1 M NaNO<sub>3</sub> at a concentration of 15 mg/ml for 2–3 days at room temperature and filtered through a 0.45  $\mu$ m syringe filter (GHP Acrodisc 13, Pall Corp., Ann Arbor, MI, USA) before injection. The flow rate and injection volume were 1 ml/min and 100  $\mu$ l, respectively.

The molar mass of samples was estimated by using pullulan standards. Pullulans with molar masses of 342, 1,320, 5,900, 11,800, 47,300, 112,000, 212,000 g/mol were used for column calibration. They were dissolved in the same eluent at a concentration of 1–2 mg/ml. The SEC data were processed with the OmniSEC 4.5 software (Viscotek Corp.).

### 2.8. Protein content

The soluble protein content in the sGGM and partitioned GGM fractions was analyzed using a Bio-Rad Protein Assay kit (Bio-Rad, USA) following the manufacturer's instructions at 595 nm. Five concentrations of BSA were used as standards. The standards and samples were dissolved in the same buffer used in the emulsions.

## 3. Results

### 3.1. Emulsion appearance

We varied the rapeseed oil content in emulsions at 0.5, 1, 2.5, 5, and 10 wt% stabilized by 1 wt% purified (ethanol-precipitated) GGM. Consequently, emulsions at GGM to oil ratios of 2, 1, 0.4, 0.2, and 0.1 were obtained. Thus, the amount of continuous phase decreased as the amount of oil phase increased, keeping the total weight of the emulsions constant. The purpose was to characterize the effects of interfacial adsorption of GGM on the stability and breakdown mechanisms of emulsions and understand the correlation between emulsion component ratios and stability.

The starting GGM (sGGM) solutions (picture not shown) and the two emulsions with the highest GGM to oil ratio (2 and 1) were brownish in color (Fig. 2). As the ratio decreased, emulsions appeared white and opaque. Their consistency increased as the



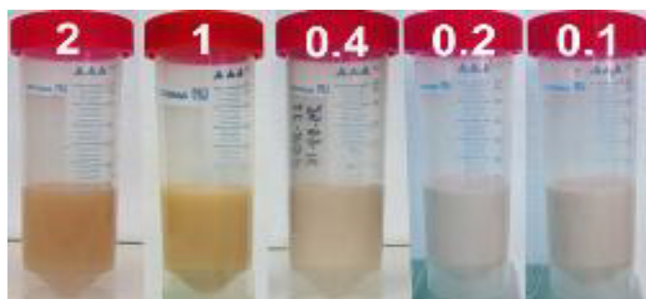


Fig. 2. Emulsions at GGM to oil ratio (2–0.1, left to right) on the preparation day.

GGM to oil ratio decreased viz. increasing oil and decreasing amount of water in the formulation.

During the accelerated storage test, at the two highest GGM to oil ratios (2 and 1), virtually no separation was visually observed. Emulsions at ratios 0.2 and 0.4 showed some creaming, but the cream layer height could not be accurately measured due to the presence of a turbid emulsion layer beneath the cream. At the lowest GGM to oil ratio (0.1), the emulsion started to separate by creaming in the first week. It separated to a distinct cream layer and a hazy serum layer by the end of experimental storage time of 9 weeks.

### 3.2. Droplet size distribution

The volume-based droplet size distribution (DSD) measurement of emulsions was followed at different time intervals from the preparation day until 9 weeks to monitor time-dependent changes in their droplet size. The changes in  $D[3,2]$  and  $D[4,3]$  that are sensitive to the presence of small-sized and large-sized droplets in emulsions, respectively, were monitored.  $D[3,2]$ ,  $D[4,3]$ ,  $D[10]$ ,  $D[50]$ , and  $D[90]$  values can be found in the supplementary materials (table S 1).

On the preparation day, all the emulsions exhibited bi- or trimodal distribution, except at GGM to oil ratio 2. The emulsions with the three highest GGM to oil ratios (2, 1 and 0.4) showed a  $D[3,2]$  of 50–70 nm, while at the two lowest ratios, 0.2 and 0.1, the  $D[3,2]$  were 300 and 850 nm, respectively. During accelerated storage at 40 °C, changes to the size of smaller droplets were not significant at ratios 2, 1 and 0.4, while at ratio 0.2, changes were observed after a week of storage and at ratio 0.1, changes to large droplets were observed already one hour after the preparation. Major changes in the droplet size were observed from the preparation day to 1–2 weeks of storage, afterward, size of large droplets continued to increase while the smaller droplets maintained their size.

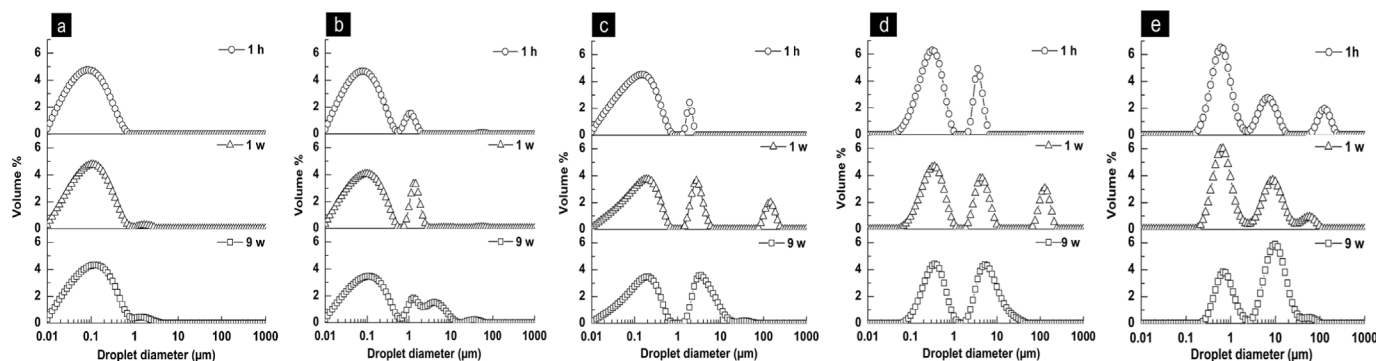


Fig. 3. DSD of emulsions at GGM to oil ratios of 2 (a), 1 (b), 0.4 (c), 0.2 (d), and 0.1 (e) after 1 h (h) of preparation, 1 and 9 weeks (w) during accelerated storage at 40 °C.

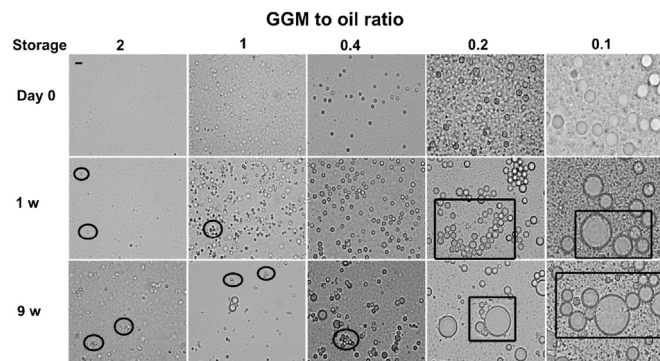


Fig. 4. Optical microscopy of emulsions at GGM to oil ratio (2–0.1, left to right) as fresh, after 1 week (w), and after 9 w during accelerated storage at 40 °C. Example of “Closed” and “open” flocs indicated by circle and rectangle, respectively. Scale bar – 5  $\mu$ m.

Based on DSD, emulsion stability depended on the GGM to oil ratio. The average droplet size increased as the GGM to oil ratio decreased in emulsion. Emulsion droplets at the three highest ratios were relatively stable during storage.

### 3.3. Optical microscopy

DSD measurement involves dilution of samples while stirring with an in-fitted rotor at a certain speed that can largely disintegrate weakly associated droplets and information on the original morphology of the droplets can be lost. A combination of DSD method and optical microscopy was used to study the possible breakdown mechanisms in emulsions (coalescence and flocculation) during storage. For microscopic sampling, there was a gentle mixing of emulsions for representative sampling; however, mixing was not too vigorous to break the association of droplets.

On the preparation day, individual droplets were observed in all emulsions at five ratios that increased in size as the GGM to oil ratio decreased in emulsions (Fig. 4, Day 0, left to right). After a week, the number of large droplets and their size increased (Figs. 4 and 1 w, left to right). Flocs were apparent in emulsions, and the nature of the flocs appeared to be different with changing ratios. Two types of flocs were generally observed: one with a compact association (“closed” flocs) and another with an open association (“open” flocs). At GGM to oil ratios 2 and 1, closed flocs (<5  $\mu$ m) were few in the first week and they continued to form progressively. At GGM to oil ratio 0.4, mostly open flocs were visible in the first week and both closed and open flocs were observed from second week onward. At GGM to oil ratio 0.2, flocculation was significant in the first week. They were mostly open, with evenly sized droplets forming a

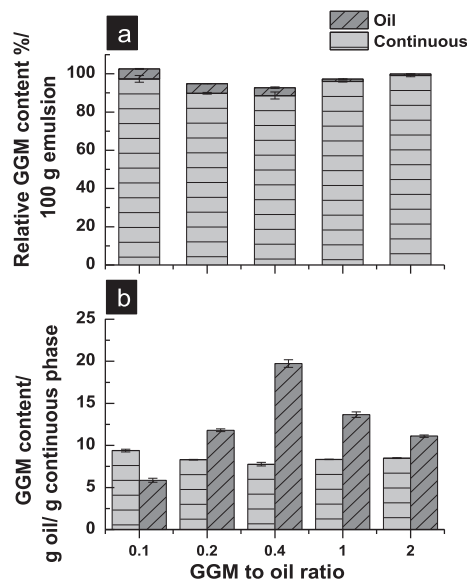


Fig. 5. (a) GGM content in the oil and continuous phase relative to GGM content in starting material at 1 wt% of a 100 g emulsion. (b) GGM content (mg) in 1 g of oil and 1 g of continuous phase. The error bars indicate standard deviation from three analyses.

grape-like appearance. As storage progressed, more closed flocs were observed with uneven droplets. At GGM to oil ratio 0.1, from the first week of storage, open flocs formed from unevenly sized droplets was observed, which continued throughout the storage. The droplets' coalescence was evident at this ratio.

#### 3.4. Distribution of GGM and interfacial properties of emulsions

From the phase partitioning of emulsions, we obtained adsorbed and unadsorbed fractions in the interface and continuous phase, respectively. The amount of GGM in the collected fractions was determined from total polysaccharide content by gas chromatography after acid methanolysis.

Once again, it is important to note that not only the GGM to oil ratio changed in emulsions. The ratio between the amounts of the continuous phase and oil decreased as the GGM to oil ratio increased, keeping the total weight of the emulsion constant. Thus, in Fig. 5a, GGM content in oil and continuous phase of a 100 g

emulsion is presented. The GGM content in each phase is relative to that of 1 wt% of GGM in the emulsion. It shows that a large proportion of GGM (more than 90%) remained in the continuous phase of emulsions and a smaller proportion adsorbed at the interface. Considering absolute weight, most GGM was associated with the oil phase at ratio 0.1 and the amount of adsorbed GGM decreased with increasing GGM to oil ratio (Fig. 5a). In contrast, Fig. 5b shows the amount of adsorbed GGM per gram of each phase and visualizes the fact that GGM was concentrated at the interfacial phase at other GGM to oil ratios except 0.1. Thus, GGM shows a tendency to adsorb at the interface, and the adsorbed amount depends on the emulsion composition.

The GGM content in Fig. 5a and b was calculated on the bulk weight of the oil phase in emulsion. In contrast, Fig. 6 presents the distribution of GGM in emulsions based on their interfacial area. Emulsion at the highest GGM to oil ratio (2) and the lowest GGM to oil ratio (0.1) created the smallest interfacial area, whereas emulsion with an intermediate GGM to oil ratio 0.4 created the largest interfacial area. However, the number of droplets were the highest at the three highest GGM to oil ratios (2, 1, and 0.4). Thus, the combined effects of the oil content (number of droplets) and droplet size determined the interfacial area in emulsions. Owing to the droplet size of tens of nanometers, many small-sized droplets created the largest interfacial area at the three highest GGM to oil ratios, whereas fewer large-sized droplets created the interface of emulsions at the two lowest GGM to ratios. The number of droplets in emulsions determined the average surface load. The average surface load was 0.09, 0.12, 0.21, 0.54, and 0.75 mg/m<sup>2</sup> at the GGM to oil ratios 2, 1, 0.4, 0.2, and 0.1, respectively. The average surface load decreased sharply from 0.75 to 0.21 mg/m<sup>2</sup> as the GGM to oil ratio increased from 0.1 to 0.4; afterward, the change in surface load was steady. The change in average surface load was inversely proportional to the change in number of droplets in emulsions.

The interfacial properties of emulsions were highly dependent on the average droplet size. Emulsions at the three highest GGM to oil ratios (0.4, 1, and 2) were similar regarding surface load and number of droplets compared to the emulsions at the two lowest GGM to oil ratios (0.1 and 0.2).

#### 3.5. Molar mass analysis

The molar mass analysis was performed to understand the differences in molar mass between adsorbed and unadsorbed GGM at different GGM to oil ratios. An aqueous-based eluent was chosen over DMSO due to the solubility problem of the partitioned fractions, and it represented the properties of GGM in emulsions, where the continuous phase is aqueous.

The color of aqueous solutions of the partitioned fractions from the interface appeared transparent, pale yellow to dark brown in water similar to that of the starting material (sGGM), whereas the unadsorbed fraction appeared white and hazy which was not clear even after filtration before injection in SEC. Due to the color differences between samples, the UV signals were studied in addition to RI signals. Absolute molar mass determination was not possible due to fluorescence from the samples that interfered with the light scattering signals, thus molar mass was estimated with pullulan standards.

To understand whether sample preparation techniques (see section 2.5) affected the macromolecular properties of GGM, a 1 wt % sGGM solution was run without the addition of rapeseed oil. sGGM showed a unimodal size distribution of RI intensity, but after homogenization, the unimodal distribution changed to trimodal (hGGM) (supplementary materials, fig S1). The main peak in the unimodal distribution of sGGM with  $M_p$  at 5300 g/mol remained in the trimodal distribution of hGGM, with an additional two peaks

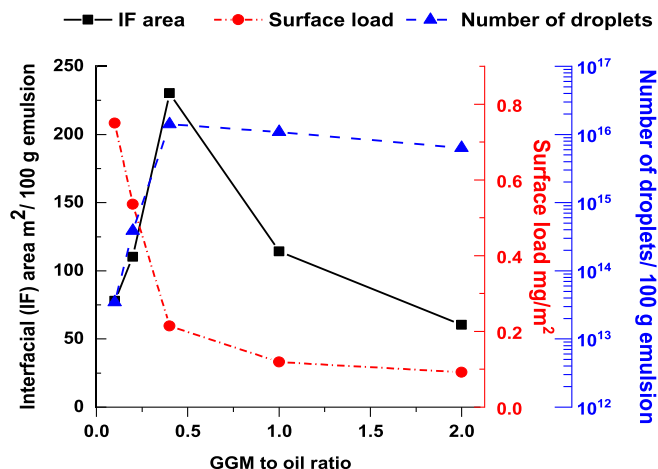


Fig. 6. Total interfacial (IF) area (m<sup>2</sup>) and number of droplets in a 100 g emulsion and the surface load (mg/m<sup>2</sup>) at five GGM to oil ratios.

with  $M_p$  of 1400 and 342 g/mol. This indicated the abundant presence of smaller-sized fractions in hGGM. Further process steps after homogenization had no effect on the distribution.

As in hGGM, the adsorbed and unadsorbed fractions from the interface and continuous phase, respectively, showed a trimodal distribution of RI intensity (Fig. 7). Molar mass was calculated from the first two peaks at the lower retention volume excluding the third peak, as it was below the lowest pullulan standard (342 g/mol). Interestingly, the RI profile of the adsorbed fraction appeared very similar to its UV profile. There were more variations in the retention time of the first peak than second; thus, both peaks were also integrated separately to have more insight on that particular fraction in the sample.

Based on the RI peak areas of the first and second peaks in the adsorbed fraction, the peak area of the first peak was higher than the second peak at all GGM to oil ratios, except ratio 2. The molar mass of the second peak was around 1600–2000 g/mol in both fractions at all ratios. The first peak of the adsorbed fraction had a slightly higher molar mass than the unadsorbed fraction at all GGM to oil ratios except ratios 2 and 1 (Table 1). At ratio 0.4, it was the highest of all which could be due to aggregation of samples. An aggregated part of approximately 720,000 g/mol can be observed (indicated by arrows in Fig. 7) however this part was not included for the molar mass calculation as it was not observed in the repeated experiment at this ratio. Similarly, the adsorbed fraction contained a significantly higher amount of very small-sized fractions below 342 g/mol than the unadsorbed fraction.

Molar mass analysis indicated a small difference in the molar mass between the adsorbed and unadsorbed fractions, especially at

the lower GGM to oil ratio. However, samples were filtered prior to SEC analysis; thus, some large-sized fractions and aggregates may have been removed during filtration.

### 3.6. Protein content

There was a very small amount of protein in both the starting material and partitioned fractions. The protein content in the sGGM and adsorbed and unadsorbed fractions from emulsions were 0.8, 1.2, and 0.9 wt%, respectively.

## 4. Discussion

### 4.1. PHWE GGM as novel wood-based food hydrocolloids

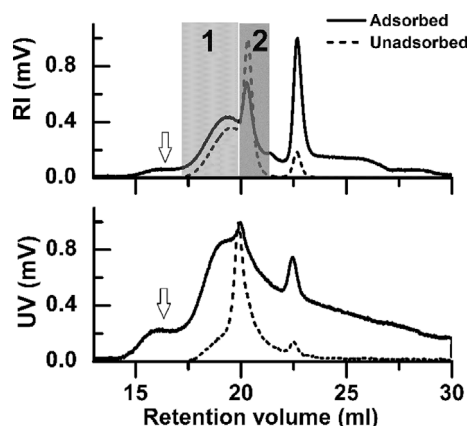
GGM are an abundant source of renewable hydrocolloids that can be established as novel emulsifiers and stabilizers in the food industry. In terms of feasibility and sustainability, PHWE is a promising aqueous-based method to extract GGM at a higher yield (about 80 wt% from spruce sawdust) on a large scale prior to pulping (Kilpeläinen et al., 2014).

Lignin-derived phenolic residues in GGM have been highly hypothesized to contribute to emulsion stabilization. To eliminate the effect of free phenolic compounds and other extractives and study the role of the polysaccharide-enriched fraction, GGM purified from free phenolics and other non-polysaccharide extractives by ethanol precipitation showed 87 wt% total carbohydrates during monosaccharide analysis. To study the stability and breakdown mechanisms of emulsions with the purified compounds, rapeseed oil was stripped to remove the possible effect of any surface-active species in stabilization.

GGM could emulsify rapeseed oil at different ratios, and the storage test at an accelerated temperature of 40 °C indicated the stability and breakdown mechanisms, as discussed in the following chapters.

### 4.2. Emulsification properties of GGM and the interfacial properties of emulsions

GGM exhibited good to excellent emulsification at all five GGM to oil ratios. The average droplet size after emulsification depended on the GGM to oil ratio. In the previous study, D[3,2] remained almost similar with 5–25 wt% oil content at a standard GGM to oil ratio of 0.2 (Mikkonen, Merger et al., 2016), whereas in the present study, D[3,2] increased with decreasing GGM to oil. The observed increase in average droplet size and the poly-dispersity of emulsions with a decreasing GGM to oil ratio was likely due to an increase in collision frequency causing coalescence due to incomplete saturation of newly formed surfaces inside the homogenizer. This indicates that there could be a fixed amount of “active” GGM that



**Fig. 7.** Normalized RI and UV intensity profiles of adsorbed and unadsorbed fractions from emulsion at GGM to oil ratio 0.4. Integration limits of the first peak and second peak indicated by shaded parts 1 and 2, respectively. The aggregated part in adsorbed fraction indicated by arrows.

**Table 1**

Estimated weight-average molar mass ( $M_w$ ) and peak maximum molar mass ( $M_p$ ) of adsorbed (AD) and unadsorbed (uAD) fractions in emulsions at five GGM to oil ratios, homogenized GGM (hGGM), and starting GGM (sGGM). Molar mass values are averaged from duplicates.

GGM to oil ratio	$M_w$ (1st & 2nd peak)		$M_w$ (1st peak)		$M_p$ (1st peak)		Ratio of peak area (1st/2nd)	
	AD	uAD	AD	uAD	AD	uAD	AD	uAD
2	7900	7600	14,800	14,700	5800	5700	0.93	0.80
1	8200	7900	14,800	14,100	5700	5300	1.12	0.80
0.4	13,000	7600	21,500	14,700	7300	5700	1.48	0.82
0.2	9300	7100	15,800	13,700	6500	5200	1.19	0.81
0.1	9800	7300	16,800	13,700	6000	5300	1.19	0.86
hGGM	8900		17,100		5400		0.91	
sGGM	12,300		n/a		5300		n/a	



are adsorbed at the interface, supported by the fact that a large proportion of GGM remained in the continuous phase of emulsions and the higher surface load at a lower GGM to oil ratio and vice-versa (Fig. 5a), however, this could be partly due to the large size of adsorbed GGM; this will be discussed in chapter 4.3. It is also expected due to a smaller interfacial area and lower number of droplets, as discussed in the following paragraph. Additionally, other factors like a large amount of oil at a lower GGM to oil ratio possibly increased the viscosity of the system that made droplet disruption difficult at a constant energy of emulsification (Jafari, Assadpoor, He, & Bhandari, 2008).

Accumulation of GGM at the droplet interface was reported previously (Mikkonen, Xu et al., 2016), the present work showed that the uneven distribution of GGM between the oil and continuous phase depended on the GGM to oil ratio (Fig. 5a), with a maximum 10% of added GGM being adsorbed at the interface. Although the oil phase represented a small fraction of the total emulsion, it was rich in GGM when considered in relation to the phase proportions, except at the lowest GGM to oil ratio (0.1) due to the largest amount of oil and the smallest amount of continuous phase (section 3.4). The average droplet size was the key to determining the interfacial properties of these emulsions and the emulsion stability, further discussed in chapter 4.4.

#### 4.3. Macromolecular properties of GGM

In general, large macromolecular hydrocolloids have an average molar mass ranging from a few to hundreds of thousands g/mol. The weight-average molar mass of sGGM in the present study was 12,000 g/mol, which is similar to that reported by Mikkonen, Merger et al. (2016) from the PHWE process. This molar mass from the PHWE process is intermediate between small molecular surfactants and large macromolecules and offers advantages in certain categories of food products (e.g., dilute beverages) over emulsifiers from large macromolecules, where the optimal region between viscosity and droplet saturation concentration is difficult to find.

Two features were noted from SEC analysis results. First, based on molar mass analysis of adsorbed and unadsorbed fraction in emulsions, there was an indication on the adsorption of large-sized fractions at the interface of emulsions at GGM to oil ratios 0.4, 0.2, and 0.1, as observed previously (Mikkonen, Xu et al., 2016). During turbulent conditions in the homogenizer, the adsorption kinetics are driven by convection, where large macromolecules and aggregates are more likely to be adsorbed at the interface (Nilsson & Bergenstahl, 2007), different from static conditions, where kinetics are dominated by diffusion, however, there was also an abundant amount of very small-sized fractions adsorbed at the interface. Second, the RI and UV profiles of the adsorbed fraction were similar. Relating this to the sensitivity of RI and UV detector towards the total concentration and UV-absorbing compounds in the eluting species, respectively, adsorbed fraction at the interface possibly contain compounds that absorb at 280 nm. With a negligible amount of protein in starting material (sGGM) and partitioned fractions, the UV-absorbing compounds could be wood-derived aromatic residues, also supported by the difference in the color of the aqueous solution of the partitioned fractions. The brown color of the adsorbed fraction is expected to originate from wood lignin-derived compounds, which indicates that the phenolic residues structurally bound to the GGM chain were precipitated during the ethanol precipitation process and retained in the purified GGM (Lehtonen et al., 2016).

It is interesting to note that coalescence proceeded at a slower rate after 1 week of storage (Fig. 3). Previous studies had also well noted the stabilizing ability and hypothesized Pickering-type

stabilization in GGM emulsions (Mikkonen, Merger, et al., 2016; Mikkonen, Xu, et al., 2016). The starting GGM could remain in the aggregated state in solution, as noted previously (Parikka et al., 2010) and dissociate into small-sized fractions during homogenization. They could have re-associated and formed Pickering particles that are microparticles to supramolecular or nanoparticles (Dickinson, 2017), contributing to stabilize the interfaces. Structurally bound phenolic residues possibly aid the formation of assemblies as the reactive sites for bond formation increase, since the GGM content used in our study was in the dilute regime, as critical overlap concentration ( $c^*$ ) is 62.5 mg/ml (Mikkonen, Merger et al., 2016), concentration-induced entanglements is unlikely to occur. In addition to the stabilizing role of assemblies at the interfaces, they might also generate structural barriers preventing droplet coalescence in the continuous phase (Dickinson, 2017). Further study is planned to characterize GGM assemblies.

#### 4.4. Physical stability and breakdown mechanisms

Flocculation, a potentially reversible process, was the reason for emulsion instability in the diluted oil-in-water system at all five GGM to oil ratios, including coalescence at the two lowest ratios during accelerated storage temperature at 40 °C. Flocculation has been observed previously in GGM-stabilized emulsions (Mikkonen, Xu, et al., 2016) and can be expected for diluted emulsions stabilized alone by non-gelling polysaccharides (Dickinson, 2003). The physiochemical basis for this phenomenon is the domination of the net attraction forces over the repulsive forces between the oil droplets. Apart from Van der Waals and other hydrophobic forces, depletion forces were the major attractive forces between droplets as steric repulsions are not strong in GGM emulsions, depicted by  $\zeta$  potential of  $\approx -10$  mV (Mikkonen, Xu, et al., 2016). Depletion flocculation was the possible effect due to a large amount of unadsorbed GGM in the continuous phase of emulsions, as previously exhibited by a wide variety of biopolymers (Chanamai & McClements, 2001). This is most likely the reason for closed flocs at the higher GGM to oil ratios (droplets inside circle, Fig. 4).

At ratios 2 and 1, coalescence was not apparent in the emulsion during storage, which clearly indicates complete saturation of droplets by GGM even though the surface load was lower. The lower surface load could be due to adsorption of small-sized polysaccharides. At ratio 0.4, smaller droplets in the emulsions were relatively stable, changes occurred to large droplets in the emulsions possibly due to incomplete coverage of the droplet surface or an insufficient amount of adsorbing GGM. This is likely the reason for open flocs (droplets inside rectangle, Fig. 4) largely leading to coalescence of droplets during storage. Although ratios 0.1 and 0.2 had a higher surface load than the rest of the emulsions, coalescence was significant. As the droplet size was few hundreds of nanometers at these ratios, it is possible that the size of GGM molecules was small for the complete coverage of the droplets' surface leading to open flocs and further coalescence. Additionally, the larger-sized GGM adsorbed at the interface at lower GGM to oil ratios could aid to flocculation.

The effect of GGM to oil ratio was significant to achieve emulsion stability. The emulsions at the three highest GGM to oil ratios (0.4, 1, and 2) were the most interesting in the present study. They had similar surface load and number of droplets in emulsions. Relating this to the physical stability of emulsions at these ratios, there was possibly a monolayer adsorption of GGM with a surface load of 0.1–0.2 mg/m<sup>2</sup>. Whereas, at the two lowest GGM to oil ratios, there was either an unevenly covered droplet surface or, most likely, the adsorption of few large-sized molecules. This study demonstrates that high surface load does not necessarily indicate higher emulsion stability.



## 5. Conclusion

GGM can be extracted in kilogram scale from lignocellulosic biomass using PHWE method. Not limited to spruce GGM, this method can be used as a general concept for other wood hemicelluloses. Aqueous extraction of hemicelluloses without chemical additives makes this process sustainable, and the hemicelluloses are hypothesized as suitable for food and other applications. Information on GGM's function mechanism is important for bio-refineries regarding adjusting the extraction and isolation conditions, as well as for industries in product development. This study exhibited the functionality of GGM as excellent emulsifiers and stabilizers of oil-in-water emulsions from GGM to oil ratios 2–0.4. Stabilization of emulsions occurred by adsorbed GGM polysaccharides possibly resulting in Pickering-type stabilization. This study revealed the macromolecular properties of polysaccharides and component ratio in emulsion as important factors for obtaining desired interfacial saturation. This study helps in the optimization of emulsion formulation, and the results from the accelerated storage test can be implied to predict the stability of dilute emulsions (e.g., low-fat salad dressings, beverages) at room temperature or chilled storage. With the development in the fundamental and application studies of GGM, along with optimization of extraction methods, valorizing abundant wood bio-refinery streams for functional food hydrocolloids can be achieved.

## Conflicts of interest

There are no conflicts to declare.

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## Appendix A. Supplementary data

Supplementary data related to this article can be found at <https://doi.org/10.1016/j.foodhyd.2018.03.020>.

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